

US EPA ARCHIVE DOCUMENT

MRID No. 436781-20

DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4

1. CHEMICAL: Azoxystrobin PC Code No.: 128810

2. TEST MATERIAL: ICIA5504 Purity: 96.2%

- 3. CITATION:**

Authors: Jon E. Rhodes, Yuan Yang and David Abram
Title: Early Life Stage Toxicity of ICIA5504 to
the Fathead Minnow (*Pimephales promelas*)
Under Flow Through Conditions

Study Completion Date: August 22, 1994

Laboratory: ABC Laboratories, Inc., Columbia, Missouri

Laboratory Report ID: 41594

Sponsor: Zeneca Limited, Wilmington, DE

MRID No.: 436781-20

- 4. REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature:

W. Lakin

4/03/96

- 5. APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature:

J. T. Coven
6/21/96

6/21/46

6. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a fish early life-stage toxicity test.

Results synopsis:

Most sensitive endpoint: length

NOEC: 147 ppb LOEC: 193 ppb MATC: 168 ppb

- ## **7. ADEQUACY OF THE STUDY:**

A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

1

**DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4**

1. **CHEMICAL:** Sulfentrazone128810
PC Code No.: 1290012. **TEST MATERIAL:** ICIA5504Purity: 96.2%3. **CITATION:**

Authors: Jon E. Rhodes, Yuan Yang and David Abram
Title: Early Life Stage Toxicity of ICIA5504 to
 the Fathead Minnow (*Pimephales promelas*)
 Under Flow Through Conditions

Study Completion Date: August 22, 1994

Laboratory: ABC Laboratories, Inc., Columbia, Missouri.

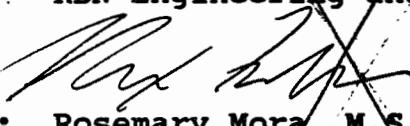
Laboratory Report ID: 41594

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MRID No.: 436781-20

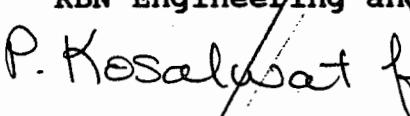
DP Barcode: D217072, D217070

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist,
 KBN Engineering and Applied Sciences, Inc.

Signature: 

Date: 1/19/96

APPROVED BY: Rosemary Mora, M.S., Toxicologist,
 KBN Engineering and Applied Sciences, Inc.

Signature: 

Date: 1/22/96

5. **APPROVED BY:**

Signature:

Date:

6. **CONCLUSIONS:** This study is scientifically sound, fulfills the guideline requirements for a fish early life-stage toxicity test, and can be classified as Core. Based on mean measured concentrations, the NOEC and LOEC for fathead minnows exposed to ICIA5504 were 147 and 193 ppb, respectively. The MATC was 168 ppb.

7. **ADEQUACY OF THE STUDY:**

A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

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2

8. MAJOR GUIDELINE DEVIATIONS:

1. The hardness of the dilution water (140-154 mg/L as CaCO₃) was higher than recommended (40-48 mg/L as CaCO₃).
2. The pH of the dilution water (8.1-8.3) was higher than recommended (7.2-7.6).

9. MATERIALS AND METHODS:**A. Biological System:**

Guideline Criteria	Reported Information
Species: A freshwater or saltwater fish species.	<i>Pimephales promelas</i>
Source: Commercial fishery, wild, or brood stock.	In-house brood stock
Age at beginning of test: Embryos 2 to 24 hours old.	≤24 hours old at initiation
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for posthatch exposure.	30 eggs/cup, 4 cups per treatment or control 20 fish/replicate, 4 replicates per treatment or control
Posthatch: % of embryos that produce live fry must be ≥ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	86.1% egg hatch in dilution water and 87.6% in solvent control. 1.2 times
Feeding: Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32.	Fish fed at least twice a day. Food was withheld 24 hours before study termination.
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Positive counts of surviving fry were made at least once every two days.

Guideline Criteria	Reported Information
Controls: Avg. survival at end of test must be $\geq 80\%$. Survival in any control chamber must not be $<70\%$.	Average of 100% and 98.8% survival in the dilution water and solvent control groups, respectively. Survival in each control group replicate was $\geq 95\%$.
Controls: Negative control and carrier control (when applicable) are required.	Dilution water and solvent control ($<0.10 \text{ mL/L}$).

Comments: None.

B. Physical System:

Guideline Criteria	Reported Information
Test Water: 1) May be natural (well or spring) or reconstituted water. 2) Water should be sterilized with UV radiation and screened for contaminants. 3) Hardness of 40-48 mg/L as CaCO_3 , pH of 7.2-7.6	1) Natural well water. 2) Water was filtered, partially sterilized with UV radiation and screened for contaminants. 3) Hardness of 140-154 mg/L as CaCO_3 , pH of 8.1 to 8.3.
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature. For fathead minnow, 25°C is recommended.	Range from 25.0 to 25.8°C.
Photoperiod: Recommend 16L/8D.	16 h light, 8 h dark
Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.	Intermittent-flow proportional diluter. Treatment concentrations of 45, 90, 140, 180, 360, and 720 $\mu\text{g/L}$.

Guideline Criteria	Reported Information
Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.	1) Mixing chamber used. 2) No aeration of exposure solutions. 3) Mixing confirmed by analysis. 4) Not reported.
Test Vessels: All glass or glass with stainless steel frame.	15.5 x 31 x 25 cm glass aquaria.
Embryo Cups: 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	9-cm diameter glass jars with 40-mesh Nitex-screen bottoms.
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain DO at above 75% of saturation and maintain the toxicant level.	7.8 volume replacements/24 h. DO and chemical concentrations confirmed by analysis.
Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.	DO \geq 80% (6.3 mg/L) of saturation at all times.

Comments: Hardness and pH of the dilution water were higher than recommended.

C. Chemical System:

Guideline Criteria	Reported Information
Concentrations: Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate. - Toxicant conc. must be measured in one tank at each toxicant level every week. - One concentration must adversely affect a life stage and one concentration must not affect any life stage.	- Yes; control, solvent control and mean measured conc. of 51.1, 96.3, 147, 193, 374, and 750 µg/L. - Yes. - Yes.
Other Variables: DO must be measured at each conc. at least once a week.	Yes, DO \geq 80% at all times.
Solvents: Should not exceed 0.1 mL/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Acetone (<0.1 mL/L).

Comments: None.**10. REPORTED RESULTS:**

Guideline Criteria	Reported Information
Data Endpoints must include: - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if approp.); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs.	Data include: - Number of eggs hatch; - Time to hatch (days 3-5); - 28-day posthatch survival; - 28-day posthatch length; - 28-day posthatch wet weight; - Morphological and behavioral observations.
Raw data included? (Y/N)	Yes.

Effects Data:

Toxicant Concentration (ppb)		Mean Percent Hatch	Percent Post-Hatch Survival (28 days)	Standard Length (mm)	Blotted Wet weight (g)
Nominal	Measured				
Control	<17	86.1	100	22.8	0.190
Solvent	<17	87.6	98.8	23.0	0.203
45	51.1	89.4	98.8	22.8	0.194
90	96.3	81.9	98.8	22.6	0.190
140	147	82.6	98.8	22.6	0.192
180	193	86.0	98.8	22.2	0.190
360	374	88.1	97.5	20.9	0.161
720	750	33.6	0	--	--

Toxicity Observations: No physical or behavioral abnormalities were observed during the study.

Statistical Results:

Statistical Method: Contingency table methods were used to analyze egg hatchability and survival data. ANOVA was used for continuous scale measurements (length and wet weight) with Dunnett's test for control comparisons.

NOEC: 147 µg/L LOEC: 193 µg/L MATC: 168 µg/L

Most sensitive endpoint: 28-day standard length

Comments: Hatch, survival, and growth data from the treatment groups were compared to data from the pooled control.

11. REVIEWER'S STATISTICAL RESULTS:

Statistical Method: ANOVA (nested) and Dunnett's test for pooled mean comparisons.

NOEC: 147 ppb LOEC: 193 ppb MATC: 168 ppb
Most sensitive endpoint: 28-day standard length

Comments: Hatch, survival, and growth data from the treatment groups were compared to the data from the pooled control. An ANOVA was performed using a nested design with vessels nested within aquaria and aquaria nested within treatments.

12. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for a fish early life-stage toxicity test using the fathead minnow. Based on mean measured concentrations, the NOEC and LOEC for fathead minnows exposed to ICIA5504 were 147 and 193 ppb, respectively. The MATC was 168 ppb. This study is classified as Core.

OBS	REP	AQUA	LENGTH	WEIGHT
1	A	A	25.2293	0.186
2	A	A	20.9299	0.243
3	A	A	25.2345	0.198
4	A	A	22.6554	0.222
5	A	A	26.6627	0.221
6	A	A	22.0560	0.235
7	A	A	24.0864	0.327
8	A	A	23.4791	0.173
9	A	A	23.8816	0.177
10	A	A	23.8615	0.203
11	A	A	22.3407	0.225
12	A	A	22.9852	0.189
13	A	A	23.6524	0.158
14	A	A	24.0787	0.187
15	A	A	24.1097	0.178
16	A	A	20.219	0.199
17	A	A	24.9022	0.249
18	A	A	22.3712	0.165
19	A	A	22.5508	0.127
20	A	A	23.5989	0.222
21	A	A	23.0075	0.182
22	A	A	24.0989	0.125
23	A	A	23.4847	0.187
24	A	A	21.9039	0.230
25	A	A	23.4768	0.142
26	A	A	22.5797	0.153
27	A	A	25.8398	0.209
28	A	A	22.9692	0.259
29	A	A	22.19756	0.194
30	A	A	22.2147	0.188
31	A	A	24.6306	0.211
32	A	A	22.0818	0.188
33	A	A	26.6074	0.270
34	A	A	22.3188	0.223
35	A	A	26.1421	0.175
36	A	A	24.8623	0.126
37	A	A	20.0783	0.207
38	A	A	22.9441	0.179
39	A	A	22.2707	0.196
40	A	A	22.3075	0.129
41	A	A	21.9451	0.234
42	A	A	24.8656	0.273
43	A	A	21.1683	0.261
44	A	A	25.0169	0.202
45	A	A	23.8496	0.198
46	A	A	24.4689	0.147
47	A	A	23.9201	0.202
48	A	A	22.3192	0.155
49	A	A	20.9408	0.110
50	A	A	23.7882	0.159
51	B	B	ccc	ccc
52	B	B	cccc	cccc
53	B	B	cccc	cccc
54	B	B	cccc	cccc
55	B	B	cccc	cccc
56	B	B	cccc	cccc

The SAS System 09:15 Friday, December 1, 1995

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OBS	REP	AQUA	LENGTH	WEIGHT
57	C	B	21.4116	0.208
58	C	B	19.7697	0.169
59	C	B	21.3046	0.158
60	C	B	21.5777	0.187
61	D	B	21.7545	0.151
62	D	B	25.3912	0.262
63	D	B	20.6534	0.134
64	D	B	22.0696	0.115
65	D	B	20.5812	0.230
66	D	B	21.6079	0.279
67	D	B	22.2849	0.171
68	D	B	22.7104	0.087
69	D	B	20.8177	0.135
70	D	B	23.7987	0.285
71	D	B	25.2637	0.168
72	D	B	18.6360	0.231
73	D	B	22.8293	0.212
74	D	B	23.8585	0.222
75	D	B	20.0000	0.161
76	D	B	25.5458	0.114
77	D	B	21.8389	0.135
78	D	B	24.1151	0.274
79	D	B	26.1744	0.169
80	D	B	20.7379	0.163

OBS	REP	AQUA	LENGTH	WEIGHT
81	E	C	24.6412	0.219
82	E	C	27.0560	0.179
83	E	C	22.9636	0.221
84	E	C	22.6717	0.312
85	E	C	23.2777	0.255
86	E	C	23.8506	0.198
87	E	C	23.2903	0.188
88	E	C	23.0618	0.201
89	E	C	24.1542	0.171
90	E	C	23.7231	0.223
91	E	C	22.5768	0.250
92	E	C	21.4396	0.184
93	E	C	24.5271	0.243
94	E	C	24.6936	0.223
95	E	C	22.2250	0.201
96	E	C	24.3634	0.254
97	E	C	22.7018	0.185
98	E	C	21.9455	0.201
99	E	C	22.6099	0.246
100	E	C	24.3677	0.185
101	E	C	22.5634	0.188
102	E	C	19.5334	0.211
103	E	C	22.4789	0.214
104	E	C	24.6330	0.244
105	E	C	22.3552	0.186

The SAS System 09:15 Friday, December 1, 1995

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OBS	REP	AQUA	LENGTH	WEIGHT
106	F	C	22.1168	0.171
107	F	C	23.9768	0.102
108	F	C	20.9256	0.178
109	F	C	23.2119	0.168

9

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C - Control
V - Vehicle Control

General Linear Models Procedure
Class Level Information

TRT	Class	Levels	Values
REP		2	C V
AQUA		4	A B C D

Number of observations in data set = 159

The SAS System 09:15 Friday, December 1, 1995 6

General Linear Models Procedure

Dependent Variable: LENGTH	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
	Source	7	23.00469091	3.28638442	0.98	0.4442
	Model					
	Error	151	506.51704250	3.35441750		
Corrected Total	R-Square	158	529.52173341			
	C.V.			Root MSE	LENGTH Mean	
	0.063444		8.004399	1.8315069	22.881255	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	1.84185256	1.84185256	0.55	0.4598
AQUA(TRT)	2	14.09709980	7.04854990	2.10	0.1259
REP(TRT*AQUA)	4	7.05753655	1.76643464	0.53	0.7163
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	1.83008972	1.83008972	0.55	0.4613
AQUA(TRT)	2	13.8334936	6.91672468	2.06	0.1308
REP(TRT*AQUA)	4	7.05753855	1.76643464	0.53	0.7163

The SAS System 09:15 Friday, December 1, 1995

General Linear Models Procedure

Dependent Variable: WEIGHT	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
	Source	7	0.02165756	0.00312251	1.44	0.1947
	Model					
	Error	151	0.32827574	0.00217401		
Corrected Total	R-Square	158	0.35013330			
	C.V.			Root MSE	WEIGHT Mean	
	0.062426		23.75485	0.0466263	0.1964465	

The SAS System 09:15 Friday, December 1, 1995

10

No significant ($P < 0.05$) between Control & Vehicle
CONT'D.

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	80	22.7743013	1.8666718	18.6360000	26.6627000
HEIGHT	80	0.1904125	0.0476729	0.0870000	0.3270000

TRT=V

Source	DF	Type I SS	Mean square	F Value	Pr > F	LENGTH Mean
TRT	5	231.43513151	46.28702630	11.31	0.0001	17
AQUA(TRT)	8	43.11264083	5.38908010	1.32	0.2323	18
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953	19
Source	DF	Type III SS	Mean Square	F Value	Pr > F	Mean
TRT	5	231.22299551	46.24459990	11.30	0.0001	20
AQUA(TRT)	8	42.76878186	5.34689773	1.31	0.2376	21
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953	22

The SAS System 09:15 Friday, December 1, 1995

General Linear Models Procedure						
Dependent Variable: WEIGHT		Sum of Squares	Mean Square	F Value	Pr > F	WEIGHT Mean
Source	DF					
Model	27	0.11263924	0.00417182	1.72	0.0140	35
Error	523	1.26687493	0.00242232			36
Corrected Total	550	1.37951417				37
R-Square		C.V.	Root MSE			38
0.081651		26.11729	0.0492171	0.1884465		39
Source	DF	Type I SS	Mean Square	F Value	Pr > F	40
TRT	5	0.07430602	0.01486120	6.14	0.0001	41
AQUA(TRT)	8	0.01842720	0.00230340	0.95	0.4739	42
REP(TRT*AQUA)	14	0.01990603	0.00142186	0.59	0.8761	43
Source	DF	Type III SS	Mean Square	F Value	Pr > F	44
TRT	5	0.07412713	0.01482543	6.12	0.0001	45
AQUA(TRT)	8	0.01824119	0.00228015	0.94	0.4818	46
REP(TRT*AQUA)	14	0.01990603	0.00142186	0.59	0.8761	47

The SAS System 09:15 Friday, December 1, 1995

Obs	REP	AQUA	LENGTH	WEIGHT	Obs	REP	AQUA	LENGTH	WEIGHT
1	A	A	25.2293	0.186	57	C	B	21.4116	0.208
2	A	A	20.9299	0.243	58	C	B	19.7697	0.169
3	A	A	25.2345	0.198	59	C	B	21.3046	0.138
4	A	A	22.4554	0.222	60	C	B	21.5777	0.187
5	A	A	26.6627	0.221	61	D	B	21.7545	0.151
6	A	A	22.0560	0.235	62	D	B	23.3912	0.262
7	A	A	24.0864	0.327	63	D	B	20.6534	0.134
8	A	A	23.4791	0.173	64	B	B	22.0696	0.115
9	A	A	21.8816	0.177	65	B	B	20.5812	0.230
10	A	A	23.8615	0.203	66	B	B	21.6079	0.279
11	A	A	22.3407	0.225	67	B	B	22.2849	0.171
					68	B	B	22.7104	0.087
					69	B	B	20.8177	0.135
					70	D	B	23.7987	0.285
					71	D	B	25.2637	0.168
					72	D	B	18.6360	0.231

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The SAS System 09:15 Friday, December 1, 1995

Obs - Observation
 Rep - Replicates
 AQUA - Agvarian

12

OBS	REP	AQUA	LENGTH	WEIGHT
100				
101				
102				
103				
104				
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106				
107				
108				
109				
110				
111				
112	F	C	19.1818	0.245
113	F	C	26.2058	0.211
114	F	C	24.2372	0.152
115	F	C	21.9752	0.280
116	F	C	25.7420	0.212
117	F	C	23.0361	0.173
118	F	C	20.5812	0.136
119	F	C	24.3864	0.280
120	F	C	22.7046	0.176
121	F	C	22.0387	0.174
122	F	C	22.0161	0.190
123	F	C	21.4719	0.157
124	F	C	21.4666	0.173
125	F	C	22.4825	0.174
126	F	C	22.0387	0.202
127	F	C	22.1165	0.162
128	F	C	22.5721	0.198
129	F	C	0.0000	0.107
130	F	C	0.0000	0.190
131	F	C	0.0000	0.246
132	F	C	0.0000	0.190
133	F	C	0.0000	0.190

TRT=C
(continued)

OBS	REP	AQUA	LENGTH	WEIGHT
100				
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The SAS System
09:15 Friday, December 1, 1995

15

OBS	REP	AQUA	LENGTH	WEIGHT
160	A	A	23.7231	0.2
161	A	A	23.7341	0.209
162	A	A	22.6099	0.190
163	A	A	23.0840	0.157
164	A	A	21.8474	0.142
165	A	A	22.7039	0.214
166	A	A	24.2332	0.225
167	A	A	24.5718	0.184
168	A	A	21.8459	0.195
169	A	A	21.3961	0.223
170	A	A	25.9513	0.169
171	A	A	25.7690	0.193
172	A	A	24.2483	0.144
173	A	A	23.2147	0.180
174	A	A	20.9943	0.176
175	A	A	22.2111	0.169
176	A	A	21.0624	0.283
177	A	A	23.7341	0.132
178	A	A	21.0129	0.197
179	A	A	21.8075	0.295
180	A	A	21.6755	0.170
181	A	A	22.4693	0.140
182	A	A	23.1266	0.132
183	A	A	25.6122	0.195
184	A	A	22.7036	0.286
185	A	A	23.1740	0.170
186	A	A	26.4049	0.162
187	A	A	0.0000	0.233
188	A	A	0.0000	0.291

16

OBS	REP	AQUA	LENGTH	WEIGHT
154	A	A	24.9166	0.225
155	A	A	24.9166	0.121
156	A	A	24.5718	0.166
157	A	A	23.8615	0.252
158	A	A	24.4952	0.131
159	A	A	22.8517	0.283
160	A	A	17.4724	0.194
161	A	A	21.8474	0.270
162	A	A	22.9090	0.247
163	A	A	21.4503	0.204
164	A	A	20.0297	0.204

13

OBS	REP	AQUA	LENGTH	WEIGHT
189	B			22.7701
190	B			0.228
191	B			0.239
192	B			0.232
193	B			0.129
194	B			0.196
195	B			0.167
196	B			0.202
197	B			0.164
198	B			0.219
199	B			0.269
200	B			0.204
201	B			0.227
202	B			0.237
203	B			0.200
204	B			0.257
205	B			0.247
206	B			0.251
207	B			0.252
208	B			0.253
209	B			0.254
210	B			0.255
211	B			0.256
212	B			0.257
213	B			0.258
214	B			0.259
The SAS System				
09:15 Friday, December 1, 1995				

... TRT=I
(continued)

OBS	REP	AQUA	LENGTH	WEIGHT
215	B			24.7666
216	C			22.5792
217	C			23.1667
218	C			0.139
219	C			21.8087
220	C			0.202
221	C			21.4291
222	C			0.167
223	C			0.160
224	C			0.220
225	C			0.254
226	C			0.143
227	C			0.200
228	C			0.232
229	C			0.270
230	C			23.1013
231	C			24.7666
232	C			22.5792
233	C			23.1667
234	C			0.139
235	C			21.8087
236	C			0.202
237	C			0.167

TRT=II

OBS	REP	AQUA	LENGTH	WEIGHT
215	B			22.7701
216	B			0.228
217	B			0.239
218	B			0.232
219	B			0.196
220	B			0.167
221	B			0.202
222	B			0.227
223	B			0.237
224	B			0.247
225	B			0.248
226	B			0.249
227	B			0.250
228	B			0.251
229	B			0.252
230	B			0.253
231	B			0.254
232	B			0.255
233	B			0.256
234	B			0.257
235	B			0.258
236	B			0.259
237	B			0.260
238	A			23.0971
239	A			0.216
240	A			0.117
241	A			0.225
242	A			0.226
243	A			0.227
The SAS System				
09:15 Friday, December 1, 1995				

... TRT=I
(continued)

OBS	REP	AQUA	LENGTH	WEIGHT
264	B			23.0972
265	B			21.5921
266	B			0.228
267	B			19.6194
268	B			0.150
269	B			0.202
270	B			0.207
271	B			0.209
272	B			0.210
273	B			0.211
274	B			0.212
275	B			0.213
276	B			0.214
277	B			0.215
278	B			0.216
279	B			0.217
280	B			0.218
281	B			0.219
282	B			0.220
283	B			0.221
284	B			0.222
285	B			0.223
286	B			0.224
287	B			0.225
288	B			0.226
289	B			0.227
290	B			0.228
291	B			0.229
292	B			0.230
293	B			0.231
294	B			0.232
295	B			0.233
296	B			0.234
297	B			0.235
298	B			0.236
299	B			0.237
300	B			0.238
301	B			0.239
302	B			0.240
303	B			0.241
304	B			0.242
The SAS System				
09:15 Friday, December 1, 1995				

... TRT=II
(continued)

OBS	REP	AQUA	LENGTH	WEIGHT
264	B			23.0972
265	B			21.5921
266	B			0.228
267	B			19.6194
268	B			0.150
269	B			0.202
270	B			0.207
271	B			0.209
272	B			0.210
273	B			0.211
274	B			0.212
275	B			0.213
276	B			0.214
277	B			0.215
278	B			0.216
279	B			0.217
280	B			0.218
281	B			0.219
282	B			0.220
283	B			0.221
284	B			0.222
285	B			0.223
286	B			0.224
287	B			0.225
288	B			0.226
289	B			0.227
290	B			0.228
291	B			0.229
292	B			0.230
293	B			0.231
294	B			0.232
295	B			0.233
296	B			0.234
297	B			0.235
298	B			0.236
299	B			0.237
300	B			0.238
301	B			0.239
302	B			0.240
303	B			0.241
304	B			0.242
The SAS System				
09:15 Friday, December 1, 1995				

14

OBs	REP	AQUA	LENGTH	WEIGHT
317	A	A	23.6823	0.194
318	A	A	22.6174	0.259
319	A	A	24.3677	0.219
320	A	A	21.8459	0.209
321	A	A	22.7072	0.211
322	A	A	23.1126	0.207
323	A	A	22.7929	0.186
324	A	A	23.8711	0.175
325	A	A	24.0256	0.223
326	A	A	23.4753	0.0897
327	A	A	21.8325	0.187
328	A	A	20.5337	0.171
329	A	A	22.4007	0.170
330	A	A	23.4753	0.222
331	A	A	24.5430	0.187
332	A	A	21.8325	0.171
333	A	A	22.0051	0.186
334	A	A	20.5337	0.171
335	A	A	22.4007	0.170
336	A	A	23.4753	0.222
337	A	A	24.5430	0.187
338	A	A	21.8325	0.171
339	A	A	22.0051	0.186
340	A	A	20.5337	0.171
341	A	A	22.4007	0.170
342	A	A	23.4753	0.222
343	A	A	24.5430	0.187
344	A	A	21.8325	0.171
345	A	A	22.0051	0.186
346	A	A	20.5337	0.171
347	A	A	22.4007	0.170
348	A	A	23.4753	0.222
349	A	A	24.5430	0.187
350	A	A	21.8325	0.171
351	A	A	22.0051	0.186
352	A	A	20.5337	0.171
353	A	A	22.4007	0.170
354	A	A	23.4753	0.222
355	A	A	24.5430	0.187
356	A	A	21.8325	0.171
357	A	A	22.0051	0.186
358	A	A	20.5337	0.171
359	A	A	22.4007	0.170
360	A	A	23.4753	0.222
361	A	A	24.5430	0.187
362	A	A	21.8325	0.171
363	A	A	22.0051	0.186
364	A	A	20.5337	0.171
365	A	A	22.4007	0.170
366	A	A	23.4753	0.222
367	A	A	24.5430	0.187
368	A	A	21.8325	0.171
369	A	A	22.0051	0.186
370	A	A	20.5337	0.171
371	A	A	22.4007	0.170
372	A	A	23.4753	0.222
373	A	A	24.5430	0.187
374	A	A	21.8325	0.171
375	A	A	22.0051	0.186
376	A	A	20.5337	0.171
377	A	A	22.4007	0.170
378	A	A	23.4753	0.222
379	A	A	24.5430	0.187
380	A	A	21.8325	0.171
381	A	A	22.0051	0.186
382	A	A	20.5337	0.171
383	A	A	22.4007	0.170
384	A	A	23.4753	0.222
385	A	A	24.5430	0.187
386	A	A	21.8325	0.171
387	A	A	22.0051	0.186
388	A	A	20.5337	0.171
389	A	A	22.4007	0.170
390	A	A	23.4753	0.222
391	A	A	24.5430	0.187
392	A	A	21.8325	0.171
393	A	A	22.0051	0.186
394	A	A	20.5337	0.171
395	A	A	22.4007	0.170
396	A	A	23.4753	0.222
397	A	A	24.5430	0.187
398	A	A	21.8325	0.171
399	A	A	22.0051	0.186
400	A	A	20.5337	0.171
401	A	A	22.4007	0.170
402	A	A	23.4753	0.222
403	A	A	24.5430	0.187
404	A	A	21.8325	0.171
405	A	A	22.0051	0.186
406	A	A	20.5337	0.171
407	A	A	22.4007	0.170
408	A	A	23.4753	0.222
409	A	A	24.5430	0.187
410	A	A	21.8325	0.171
411	A	A	22.0051	0.186
412	A	A	20.5337	0.171
413	A	A	22.4007	0.170
414	A	A	23.4753	0.222
415	A	A	24.5430	0.187
416	A	A	21.8325	0.171
417	A	A	22.0051	0.186
418	A	A	20.5337	0.171
419	A	A	22.4007	0.170
420	A	A	23.4753	0.222

OBs	REP	AQUA	LENGTH	WEIGHT
395	A	A	27.1933	0.244
396	A	A	23.1126	0.176
397	A	A	26.7236	0.313
398	A	A	21.8269	0.193
399	A	A	22.8322	0.192
400	A	A	22.4768	0.192
401	A	A	23.0967	0.331
402	A	A	23.8206	0.204
403	A	A	18.4522	0.193
404	A	A	21.8120	0.256
405	A	A	20.1693	0.192
406	A	A	24.7335	0.204
407	A	A	23.9435	0.235
408	A	A	22.6099	0.178
409	A	A	20.3104	0.193
410	A	A	22.7322	0.204
411	A	A	22.2045	0.192
412	A	A	22.7045	0.204
413	A	A	18.0841	0.203
414	A	A	23.2399	0.193
415	A	A	24.3677	0.196
416	A	A	23.5013	0.239
417	A	A	23.0978	0.237
418	A	A	25.2525	0.206
419	A	A	21.9366	0.198
420	A	A	21.4359	0.221

15

537	D	8	23.2116	0.262
538	D	8	21.8816	0.150
539	D	8	21.9895	0.216
540	D	8	22.6659	0.184
541	D	8	19.3922	0.212
542	D	8	23.2116	0.094
543	D	8	20.5527	0.094
544	D	8	20.0248	0.149
545	D	8	21.5536	0.134
546	D	8	20.0333	0.140
547	D	8	17.5509	0.237
548	D	8	19.2258	0.171
549	D	8	16.9205	0.171
550	D	8	21.3843	0.161
551	D	8	17.9939	0.187

The SAS System

09:15 Friday, December 1, 1995

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	6	C I II III IV V
REP	8	A B C D E F G H
AQUA	4	A B C D

Number of observations in data set = 551

The SAS System 09:15 Friday, December 1, 1995

General Linear Models Procedure

Dependent Variable: LENGTH

Independent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	313.47936170	11.61034673	2.84	0.0001
Error	523	2140.21088653	4.09218143		
Corrected Total	550	2453.69024823			

R-Square	C.V.	Root MSE	LENGTH Mean
0.127758	9.027539	2.0229141	22.408257

General Linear Models Procedure
Bonferroni (Dunn) T tests for variable: LENGTH

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 523 MSE= 4.092181

Critical Value of T= 2.94875

Comparisons significant at the 0.05 level are indicated by ***.

Simultaneous Lower Difference Simultaneous
Comparison Between Upper
Confidence Means Confidence
Limit

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	231.43313151	46.28702630	11.31	0.0001
AQUA(TRT)	8	43.11264083	5.38908010	1.32	0.2323
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	231.22299951	46.24459990	11.30	0.0001
AQUA(TRT)	8	42.76678186	5.34669773	1.31	0.2376
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953

The SAS System

09:15 Friday, December 1, 1995

Source	DF	Sum of squares	Mean square	F Value	Pr > F
TRT	5	0.07430602	0.01486120	6.14	0.0001
AQUA(TRT)	8	0.01842720	0.00230400	0.95	0.4739
REP(TRT*AQUA)	14	0.0199603	0.00142186	0.59	0.8761
Corrected Total	550	1.37951417			
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	0.07422713	0.01482453	6.12	0.0001
AQUA(TRT)	8	0.01821119	0.00228015	0.94	0.4818
REP(TRT*AQUA)	14	0.0199603	0.00142186	0.59	0.8761

General Linear Models Procedure

II

- C -1.1424 -0.3213 0.4998 0.6740 0.2812 1.2363 1.4991 2.7925 ***

- C -1.1424 -0.3213 0.6710 0.6710 0.2812 1.2363 1.5900 2.8850 ***

- C -1.1424 -0.3213 0.9521 0.9521 0.2812 0.6740 0.9522 2.6018 ***

- C -1.1424 -0.3213 0.9521 0.9521 0.3567 0.6740 0.9522 2.6018 ***

- C -1.1424 -0.3213 0.6915 0.6915 1.6467 0.9521 0.6740 2.6018 ***

- C -1.1424 -0.3213 0.4998 0.4998 0.9521 0.6740 0.9522 2.6018 ***

IV	- C	-1.6991	-0.6780	0.1431		
IV	- I	-1.5900	-0.6370	0.3143		
IV	- II	-1.3089	-0.3567	0.5954	***	
IV	- III	-1.3058	-0.3567	0.5924	***	
IV	- V	0.3378	1.2899	2.2421	***	
V	- C	-2.7925	-1.9679	-1.1433	***	
V	- I	-2.8330	-1.9278	-0.9726	***	
V	- II	-2.6018	-1.6667	-0.6915	***	
V	- III	-2.5988	-1.6466	-0.6945	***	
V	- IV	-2.2421	-1.2899	-0.3378	***	

The SAS System 09:15 Friday, December 1, 1995

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: WEIGHT
 NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 523 MSE= 0.002422
 Critical Value of T= 2.94875

Comparisons significant at the 0.05 level are indicated by ***.

Comparison	TRT	Simultaneous Difference Between Means		Simultaneous Lower Confidence Limit		Upper Confidence Limit	
		Lower Confidence Limit	Mean	Upper Confidence Limit	Mean	Upper Confidence Limit	
C	- I	-0.017847	0.002216	0.022278			
C	- II	-0.015257	0.004806	0.024868			
C	- III	-0.013277	0.006700	0.026677			
C	- IV	-0.013201	0.006776	0.026753			
C	- V	0.015782	0.035844	0.055906	***		
I	- C	-0.022278	-0.002216	0.017847			
I	- II	-0.020649	0.002590	0.025829			
I	- III	-0.018662	0.004484	0.027649			
I	- IV	-0.018606	0.004560	0.027725			
I	- V	0.010389	0.033628	0.056867	***		
II	- C	-0.024868	-0.004806	0.015257			
II	- I	-0.025829	-0.002590	0.020649			
II	- III	-0.021271	0.001894	0.025060			
II	- IV	-0.021295	0.001970	0.025136			
II	- V	0.007799	0.031038	0.054278	***		
III	- C	-0.026677	-0.006700	0.013277			
III	- I	-0.026649	-0.004484	0.018682			
III	- II	-0.025060	-0.001894	0.021271			
III	- III	-0.021195	0.001970	0.025136			
III	- IV	-0.023016	0.00076	0.023168			
III	- V	0.005979	0.029144	0.052310	***		

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 523 MSE= 0.002422

Critical Value of T= 2.94875

Comparisons significant at the 0.05 level are indicated by ***.

Quantiles(Def=5)			
100% Max	28.0153	99%	551
75% Q3	23.866	95%	Sum Wgts
50% Med	22.5762	90%	22.40826
25% Q1	21.2649	10%	Sum
0% Min	11.9047	5%	Variance
Range	16.1106	1%	4.461255
0.99	2.6011		0.69421
Mode	24.3677		279127.3

Extremes

Lowest	Obs	Highest	Obs
11.9047	307	26.7236	397
13.4881	356	26.7853	292
14.3204	316	27.056	82
15.3664	493	27.993	395
15.8884	257	28.0153	300

Variables=LENGTH

Moments

N	551	Sum Wgts	551
Mean	22.40826	Sum	99
Std Dev	4.461255	Variance	22.40826
Skewness	1.607929	Kurtosis	4.461255
USS	2453.69	CSS	2453.69
CV	0.089981	Std Mean	0.089981
T:Mean=0	0.0001	P> T	0.0001
Num <= 0	551	Num > 0	551
K(Sign)	0	P>=M	0.0001
Sign Rank	76038	P>=S	0.0001

Univariate Procedure

28

The SAS System 09:15 Friday, December 1, 1995

The SAS System 09:15 Friday, December 1, 1995

Univariate Procedure

30

Variable=LENGTH

Histogram

Boxplot





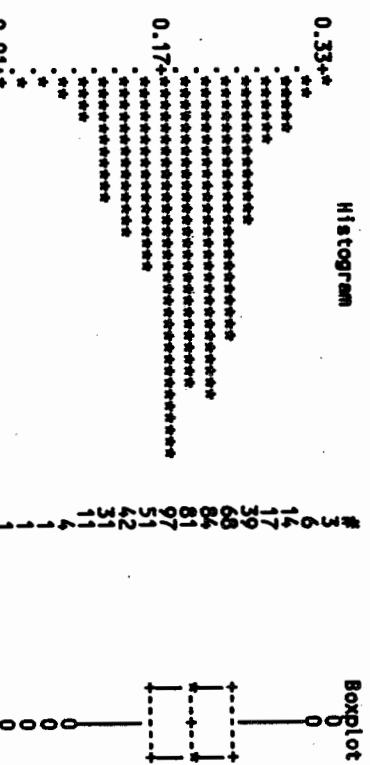
Normal Probability Plot

Extremes		
Lowest	obs	Highest
0.019(303)	0.316(
0.035(299)	0.317(
0.047(342)	0.322(
0.06(482)	0.327(
0.07(428)	0.351(

The SAS System 09:15 Friday, December 1, 1995

Univariate Procedure

Variable=WEIGHT



The SAS System 09:15 Friday, December 1, 1995
31

Variable=WEIGHT

Moments

	N	Sum Wgts	551
Mean	0.188446	Sum	834
Std Dev	0.050082	Variance	0.002508
Skewness	-0.02592	Kurtosis	0.159942
USS	20.94666	CSS	1.37514
CV	26.57625	Std Mean	0.002134
T:Mean=0	88.32467	Pr> T	0.0001
Num > 0	551	Pr>=M	0.0001
M(Sign)	275.5	Pr>=S	0.0001
Sgn Rank	76038		

Quantiles(Def=5)

	100% Max	99%	95%	90%	25%	10%	5%	1%
Range	0.312	0.331	0.221	0.188	0.158	0.125	0.105	0.07
Q3-Q1	0.063							
Mode	0.173							

19

The SAS System 10:32 Friday, December 1, 1995

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	6	C I II III IV V
REP	8	A B C D E F G H
AQUA	4	A B C D

Number of observations in data set = 551

The SAS System

10:32 Friday, December 1, 1995

General Linear Models Procedure

Note: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 523 MSE= 4.092181

Comparisons significant at the 0.05 level are indicated by ***.

Dependent Variable: LENGTH	General Linear Models Procedure					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	27	313.47936170	11.61034673	2.84	0.0001	
Error	523	2140.21088653	4.09218143			
Corrected Total	550	2453.69024823				
R-Square		C.V.	Root MSE	LENGTH Mean		
	0.127758	*9.027539	2.0229161	22.408257		

Comparison	TRT	Lower Confidence Limit	Upper Confidence Limit
I - C	-0.6790	-0.0401	0.5989
II - C	-0.9602	-0.3213	0.3177
III - C	-0.9575	-0.3213	0.3150
IV - C	-1.3142	-0.6780	-0.0417
V - C	-2.6069	-1.9679	-1.3290

The SAS System

10:32 Friday, December 1, 1995

General Linear Models Procedure

Dunnnett's One-tailed T tests for variable: WEIGHT

Note: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 523 MSE= 0.002422

Critical Value of Dunnnett's T= 2.285

Comparisons significant at the 0.05 level are indicated by ***.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
TRT	5	231.43513151	46.28702630	11.31	0.0001	
AQUA(TRT)	8	43.11264083	5.38908010	1.52	0.2323	
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953	
Source	DF	Type I SS	Mean Square	F Value	Pr > F	
TRT	5	231.43513151	46.28702630	11.31	0.0001	
AQUA(TRT)	8	42.7687186	5.34609773	1.31	0.2376	
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953	

The SAS System 10:32 Friday, December 1, 1995

General Linear Models Procedure

Comparisons significant at the 0.05 level are indicated by ***.

Comparison	TRT	Lower Confidence Limit	Upper Confidence Limit
I - C	-0.017761	-0.002216	0.013330
II - C	-0.020351	-0.004806	0.010740
III - C	-0.022179	-0.006700	0.008780
IV - C	-0.022255	-0.006776	0.008704
V - C	-0.051389	-0.035844	-0.020296

20